Dual Luciferase® Reporter Gene

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Abstract

Luciferase is an enzyme that is used in bioluminescence. Luciferase most famous form is the one found in fireflies. In this form Luciferase works in a very unique way. It binds with green fluorescent protein that is responsible for the green light emitted by the fireflies. The substrate is available for oxidation by the Luciferase and the energy that is released as a photon of green light produces the green color. For our purposes however, the Luciferase found in fireflies alone is not very relevant, but more a combination of it. Luciferase has applications in the biomedical field, as well in other biological fields such as in blood banks to tell if red blood cells are degrading. Another use is in forensic sciences where it is used in a solution that can uncover blood traces in crime scenes. It is also used in protein denaturation due to its high heat sensitivity.

Dual Luciferase

Dual reporters are commonly used to generate more accurate results when conducting an experiment. When referring to the “dual” it means that there is expression and measurement of two individual reporter enzymes occurring inside a single system at the same time. This provides much faster and better results.

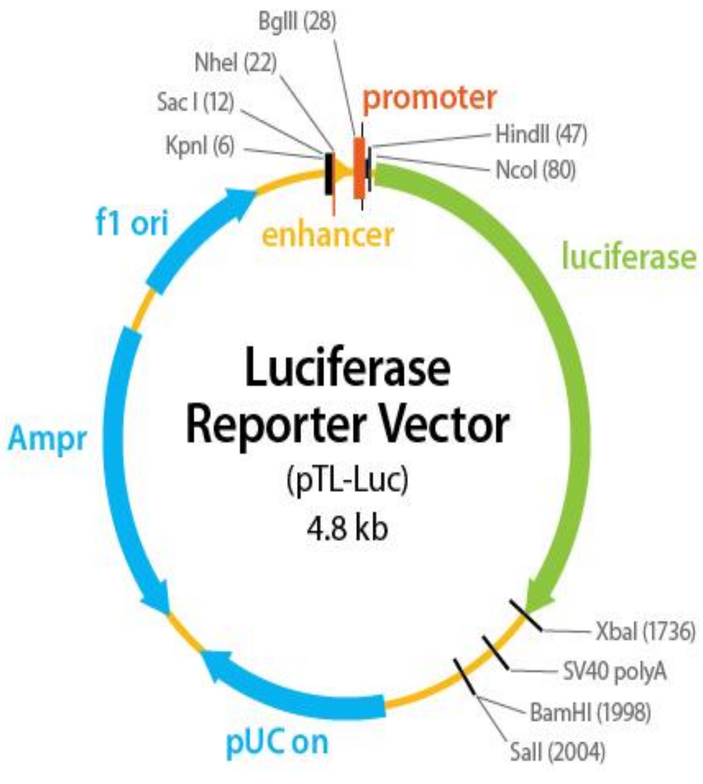

Figure 1. Vector map for Luciferase vector map

The Dual Luciferase reporter system is designed by Promega company . This dual system provides an efficient way of performing dual reporter assays. The luciferases used in this system are the firefly and renilla luciferases. The reason these two luciferases are used is because they have very unique origins and their enzymatic structure is different from each other. These chemical differences make them very important for the bioluminescent reactions.

Firefly luciferase is a monomeric protein that does not require post-translational processing for enzymatic activity. What this means is that it functions as a genetic reporter immediately upon translation. The photon emission happens because oxidation occurs and this reaction requires ATP and Mg (which generates the distinctive green color).

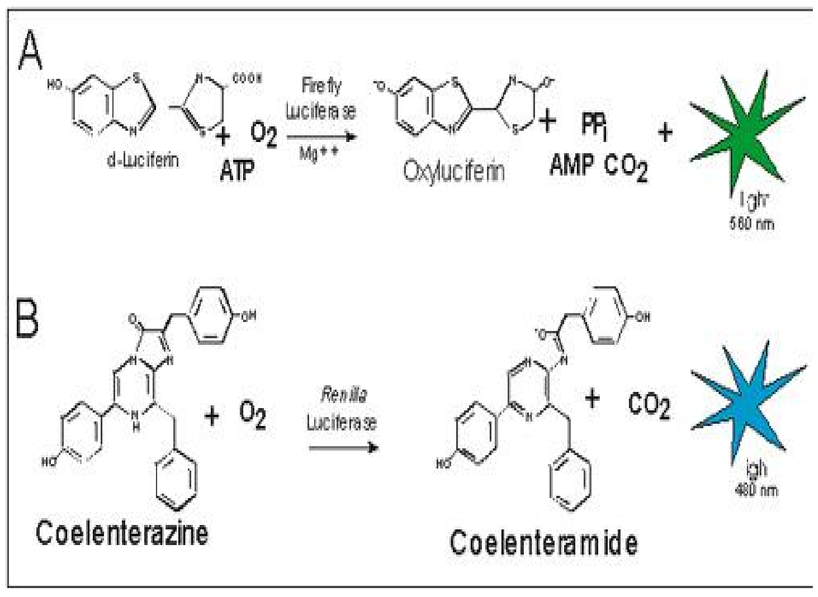

Figure 2. Bioluminescent reactions that are catalyzed by firefly and renilla luciferase. Part A shows the reactions that occurs from the firefly, producing the green light due to the combination with Mg. Part B shows the reaction that occurs with renilla luciferase.

Transfer 20ul of the solution to the luminometer, then record the activity caused by the luciferase. After this add the Stop & Glo reagent or renilla luciferase to the solution and record all activity measurement.

After following this procedure it must be determined what is the assay backgrounds. This can be found depending on the luminescence emitted in the test tubes. For firefly background luminescence can affect can affect the results. Also static electricity can alter the background of the firefly. To prevent any margin or error opaque plates must be used to prevent any light filtration. For renilla luciferase the same problems as for firefly luciferase may arise. To eliminate any errors the Stop & Glo reagent must be used as this will eliminate any autoluminescence.

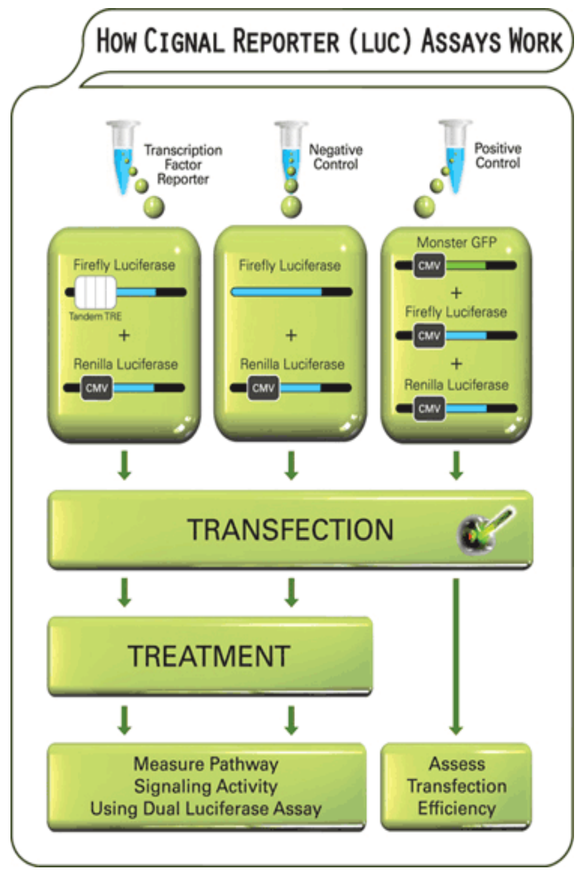

Figure 3. Steps carried out by th Cigna Qiagen company. Similar protocol to that of Promega Corporation.

Protocol

The assay for the firefly and renilla luciferase are performed one after the other using only one test tube. A luminometer, a device used to measure the amount of light emitted by a reaction is used to measure the amount of luminosity emitted when the assay is reacted. The luciferase assay reagent is added to each appropriate luminometer. Each assay is to be measured during 10 second intervals.

Conclusion

Dual Luciferase is a very important as it can yield more accurate results when conducting an experiment. There is more expression and measurement of two individual reporter enzymes. The two different types of Luciferase are very important as both the firefly and renilla can show different types of colors in response what is been looked at. Also the different applications of Luciferase from DNA markers to blood banks to solving a crime.